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ARMSTRONG LABORATORY

THE RELATIONSHIP BETWEEN STRATUM CORNEUM DIFFUSION COEFFICIENTS AND TEMPERATURE FOR HALOGENATED HYDROCARBONS

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

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The relationship between diffusion coefficients and exposure temperature was evaluated in isolated stratum corneum (SC). Due to the difficulty in measuring a diffusion coefficient directly, the movement of chemicals into and across the dermal barrier is often described as a function (the permeability constant) of the diffusion coefficient. The permeability constant also depends on the skin: chemical partition coefficient and the pathlength of diffusion. In the idealized expression of diffusion given in the Stokes-Einstein equation, diffusion is described in terms of kinetic energy and the viscous resistance of diffusion. If this relationship holds for the diffusion of halogenated chemicals in isolated stratum corneum then the observed diffusion coefficients will be dependent on the temperature of the exposure system. Rat or human stratum corneum was removed from whole thickness skin trypsin treatment and stored in a dessicator jar. Approximately 5 Mg of SC was placed into the weighing pan of a thermogravimetric analyzer and exposed to vapor or halogenated hydrocarbons. The increase in tissue weight over time for temperatures ranging from 27-40°C were evaluated. Diffusion coefficients were estimated by fitting the pre-equilibrium data with a non-steady state diffusion equation. The observed diffusion coefficients were dependent on the temperature of the exposure, indicating the importance of careful temperature control in dermal absorption studies using halogenated hydrocarbons.

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PREFACE

This technical report describes the relationship between diffusion coefficients and exposure temperature evaluated in isolated stratum corneum. Due to the difficulty in measuring a diffusion coefficient directly, the movement of chemicals into and across the dermal barrier is often described as a function (the permeability coefficient) of the diffusion coefficient. The permeability constant also depends on the skin:chemical partition coefficient and the pathlength of diffusion. In the idealized expression of diffusion given in the Stokes-Einstein equation, diffusion is described in terms of kinetic energy and the viscous resistance of diffusion. If this relationship holds for the diffusion of halogenated chemicals in isolated stratum corneum then the observed diffusion coefficients will be dependent on the temperature of the exposure system. The information contained in this report was presented in a poster format at the Society of Toxicology Annual Meeting held in Baltimore, MD in March 1995.

The animals used in this study were handled in accordance with the principles in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council, DHHS, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

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INTRODUCTION

The Stokes-Einstein Equation describes chemical diffusion as a function of temperature,

$$D = \frac{k_B T}{6\pi n r} \qquad \text{Equation 1}$$

where D is the Diffusion Coefficient, k_B is Boltzmann's constant, T is temperature, n is the solvent viscosity, and r is the molecular radius [1,2]. If the diffusional behavior of a chemical in stratum corneum is described by the Stokes-Einstein relationship, the diffusion should be proportional to temperature with a proportional constant of $k_B/6\pi nr$. The influence of temperature on measured diffusion coefficients should be accounted for in descriptions of chemical movement through the dermal barrier. This is particularly evident when viewed in the context of the dermal permeability coefficient, k_p (cm/hr), which is commonly used to describe the chemical movement in skin:

$$k_p = \frac{D \times P_{skin/chemical}}{x} \qquad \text{Equation 2}$$

where $P_{skin/chemical}$ is the skin:chemical partition coefficient, and x is the pathlength of diffusion. As can be seen in Equation 2, accurate determination of the permeability coefficient depends on the use of a diffusion coefficient determined under appropriate temperature conditions.

Thermal Gravimetric Analysis (TGA) was used to determine the diffusion coefficient for a set of halogenated hydrocarbons in rat and human stratum corneum. Diffusion coefficient versus temperature data was used to determine the quantitative relationship between temperature and diffusion.

METHODS AND MATERIALS

Materials

The chemicals of interest in this study were Dibromomethane (99% purity), Tetrachloroethylene (99.99% purity), and Chloropentafluorobenzene (99% purity). All three chemicals were purchased from Aldrich.

Method

Human cadaver skin samples examined in this study were taken from the thigh. Rat skin samples were also collected and studied from the backs of three different rats. A dermatome was utilized to remove the top 0.025 mm layer from the rat or human whole skin sample. Skin sections were carefully removed from the dermatome catch and placed on a cutting board and sliced into manageable sections approximately 1" by 1". These sections were placed onto a 0.5% solution of trypsin type II in pH 7.4 phosphate buffer-

treated filter paper, making sure the ends were not folded under, or the skin had no air bubbles underneath it. Petri dishes containing samples were placed into a Carbon Dioxide-free incubator for two hours at 37°C. Meanwhile, 100 ml of 0.005% soybean trypsin inhibitor type II-S was prepared and poured into glass dishes and an equal number of dishes was filled with distilled water. Following incubation, the top layer was carefully removed using forceps and spatula, and the stratum corneum was allowed to float on top of the trypsin inhibitor for 10 minutes. The stratum corneum was then removed and placed in the water bath for 30 minutes. The stratum corneum was removed using a wire screen and allowed to dry until all water droplets were gone. Samples were stored in a desiccator until needed.

Approximately 5 mG of rat or human stratum corneum (SC) was placed on the titanium weighing pan of the Thermal Gravimetric Analyzer (TGA) and exposed to a dry air flow of 2 L/min. Once equilibrium was reached, the dry air was simultaneously stopped and replaced with a 10,000 ppm concentration of a halogenated hydrocarbon of interest. The mass of the sample was recorded by the TGA until a chemical equilibrium was obtained and the mass appeared constant. Samples were exposed at temperatures of 27, 30, 32, 35, 37, and 40°C.

Data Analysis

Figures 1 and 2 are samples of the data obtained from the TGA, i.e. the sample mass versus time in minutes. Diffusion coefficients were estimated by fitting the pre-equilibrium data with a non-steady state diffusion equation (Equation 3) [3,4,5]. The terms in equation 3 are described below.

$$\frac{MASS}{MINF} = 1 - \exp(-\beta\, t) - \sum_{n=0}^{\infty}\left(\frac{16}{(2n+1)^2\pi^2}\right)\left(\frac{2l^2\beta}{D(2n+1)^2\pi^2 - 4l^2\beta}\right)\left(\exp(-\beta\, t) - \exp\left(-\frac{D(2n+1)^2\pi^2 t}{4l^2}\right)\right)$$

Equation 3

Description of terms in Equation 3

MASS	Mass of stratum corneum and chemical at a particular time (mg)
MINF	Mass of stratum corneum and chemical at infinite time (mg)
β	Lag constant. Equal to 0.238
t	Time (seconds)
D	Diffusion Coefficient (cm^2/sec)
l	Half thickness of the stratum corneum. Equal to 0.00034 cm

RESULTS

The diffusion coefficient was individually determined for each data set as described in the method section. Graphical results are shown in figures 1 and 2, with the tabular results of all the studies shown in table 1.

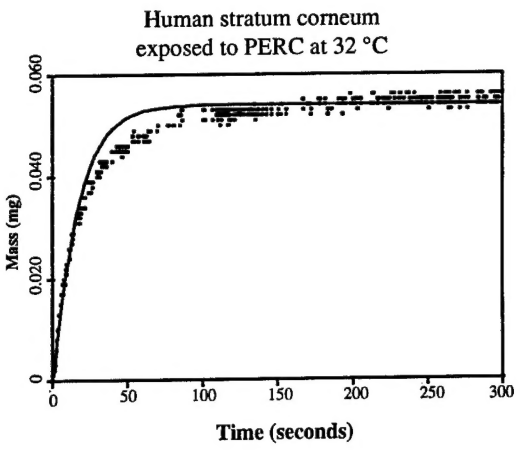

Figure 1: Example of diffusion coefficient determination of human stratum corneum at 32 °C with Tetrachloroethylene (PERC).

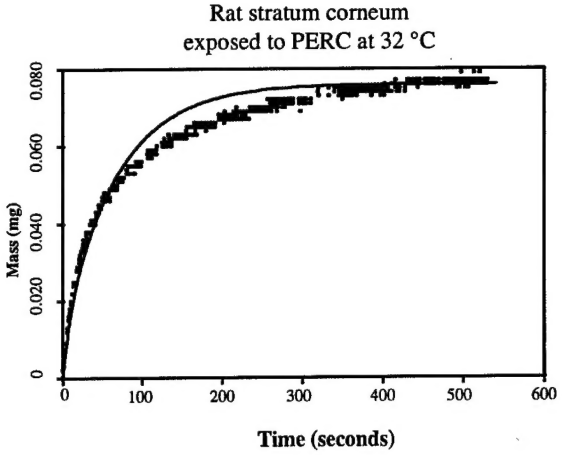

Figure 2: Example of diffusion coefficient determination of rat stratum corneum at 32 °C with Tetrachloroethylene (PERC).

Temperature (C)	CPFB Human	PERC Human	DBM Human
27	2.50e-09	2.00e-09	3.00e-09
30	3.25e-09	2.50e-09	4.00e-09
32	4.00e-09	3.00e-09	7.00e-09
35	9.00e-09	3.00e-09	8.00e-09
37	5.50e-09	5.00e-09	9.00e-09
40	7.00e-09	6.00e-09	1.50e-08

Temperature (C)	CPFB Rat (n=3)	PERC Rat (n=3)	DBM Rat (n=3)
27	4.100e-10 ± 1.493e-10	4.033e-10 ± 2.517e-11	6.333e-10 ± 5.774e-11
30	5.133e-10 ± 1.882e-10	5.233e-10 ± 2.082e-11	9.000e-10 ± 0.000e+00
32	6.333e-10 ± 1.358e-10	7.000e-10 ± 1.000e-10	1.800e-09 ± 3.000e-10
35	5.467e-10 ± 2.730e-10	9.000e-10 ± 2.646e-10	2.011e-09 ± 1.934e-11
37	8.833e-10 ± 3.753e-10	8.167e-10 ± 7.638e-11	2.133e-09 ± 2.309e-10
40	1.150e-09 ± 5.895e-10	1.233e-09 ± 3.786e-10	4.567e-09 ± 2.136e-09

Table 1: Diffusion Coefficients (cm^2/sec) for human and rat stratum corneum for Chloropentafluorobenzene (CPFB), Tetrachloroethylene (PERC), and Dibromomethane (DBM). Rat Diffusion Coefficients are an average of three different stratum corneum samples.

The rat diffusion coefficients shown are the average of the diffusion coefficients from the three different rats. The human diffusion coefficients ranged from 2.0e-9 to 1.5e-8 cm^2/sec, while the average rat diffusion coefficients were slightly lower, ranging from 4.0e-10 to 3.4e-9 cm^2/sec. A summary of these data is shown in figures 3 and 4 where the

diffusion coefficient is plotted versus temperature for each chemical and skin type. For each data set, the equation of the linear regression is shown.

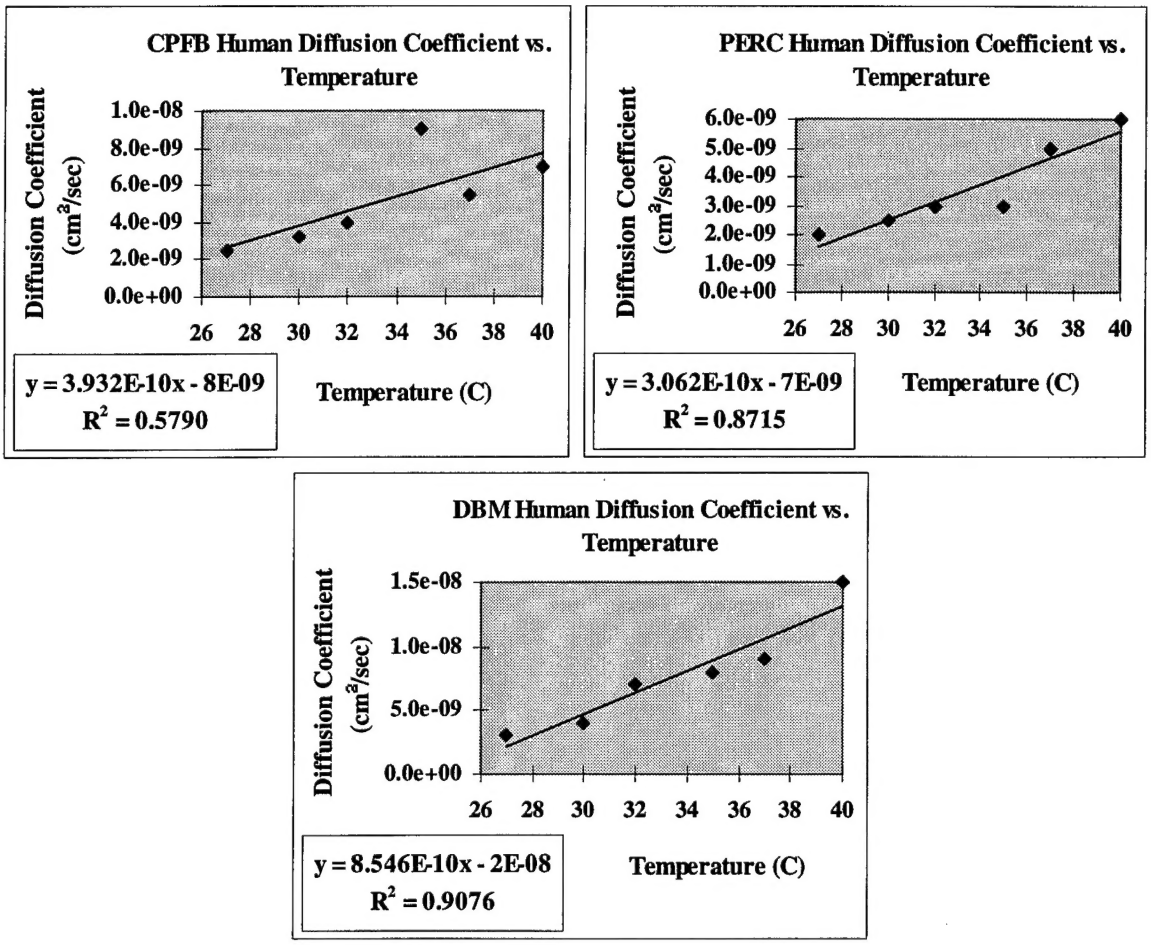

Figure 3: Plots of Human Diffusion Coefficients versus Temperature for Chloropentafluorobenzene (CPFB), Tetrachloroethylene (PERC), and Dibromomethane (DBM).

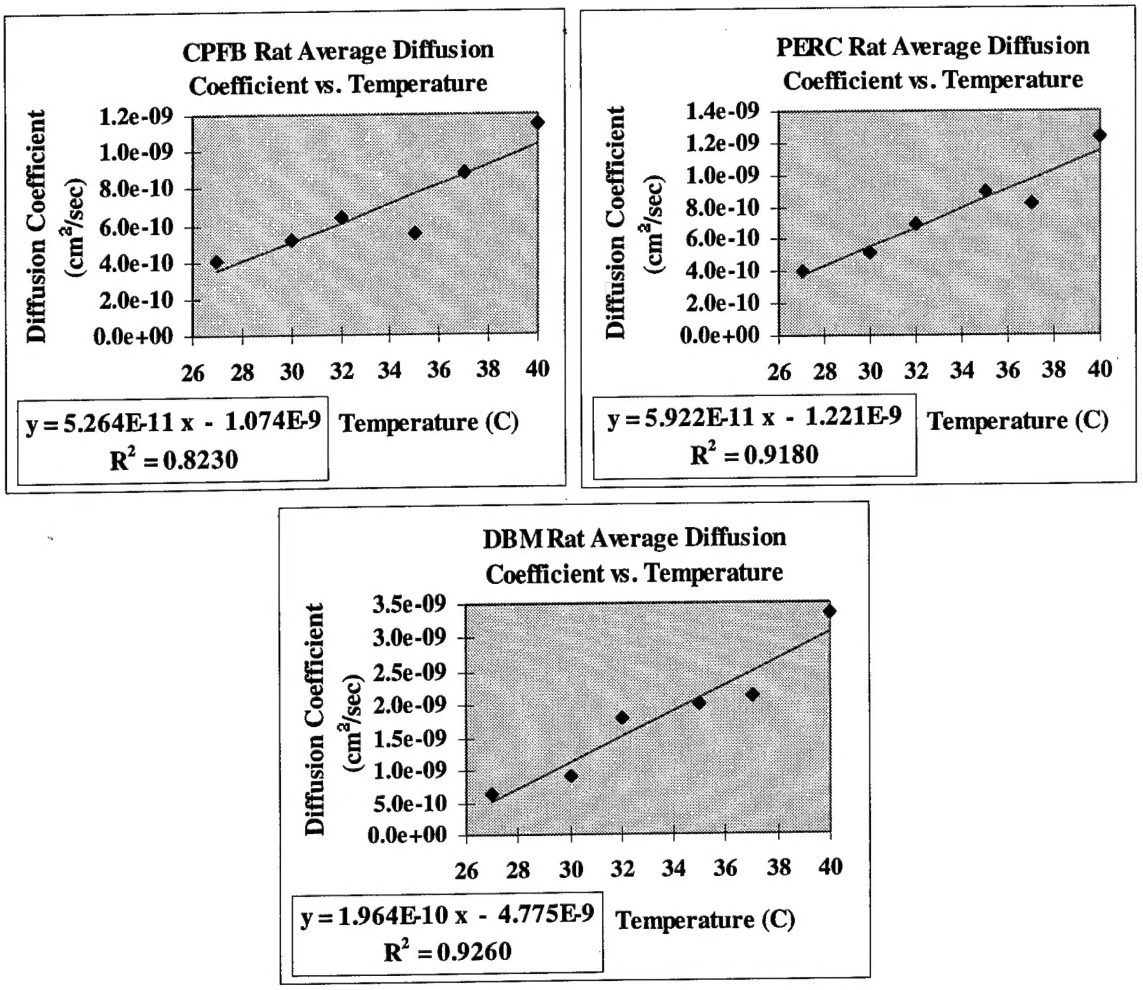

Figure 4: Plots of Rat Diffusion Coefficients versus Temperature for Chloropentafluorobenzene (CPFB), Tetrachloroethylene (PERC), and Dibromomethane (DBM).

DISCUSSION

The Stokes-Einstein equation describes chemical diffusion as directly proportional to temperature (Equation 1) with a proportionality constant of $k_B/6\pi nr$. This study clearly shows this relationship in figures 3 and 4. Since the diffusion coefficient is used to calculate the dermal permeability coefficient (Equation 2), this influence of temperature on measured diffusion coefficients should be accounted for in descriptions of chemical movement through the dermal barrier.

CONCLUSION

- Diffusion is proportional to temperature for the dermal absorption of Dibromomethane (DBM), Tetrachloroethylene (PERC), and Chloropentafluorobenzene (CPFB).

- For accurate determination of the dermal permeability coefficient, k_p, care should be taken to account for the influence of temperature on measured dermal diffusion coefficients.

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